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ENTERIC BACTERIA OF REPTILES ON JAVA AND THE KRAKATAU ISLANDS

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CONTENTS

A small survey of faecal bacteria from six species of reptile on West Java and the Krakatau Islands showed that species of *Citrobacter* were present in 17 out of 19 individual reptiles sampled. Species of *Enterobacter*, *Klebsiella* and *Pseudomonas* were also common but *Escherichia coli* was rare (present in only 2 out of 19 individual reptiles). *Streptococcus faecalis* was not detected in any reptile.

Species of *Pseudomonas* were not detected in the faeces of any of the three gecko species sampled (*Gekko gecko*, *Gekko monarchus* and *Hemidactylus frenatus*), although they were detected in samples from the other three reptile species (*Mabuia multifasciata*, *Chrysopelea paradisi* and *Varanus salvator*).

Species of *Citrobacter* and *Pseudomonas* were more common and *E. coli* less common in reptiles than in mammals (rats and bats) living in the same area.

The antibiotic-resistance patterns of the *Citrobacter* species were not significantly different between islands of the Krakatau group. *Citrobacter* species from reptiles were more resistant to chloramphenicol (43 % resistant) than those from mammals (8 % resistant). None of the isolates of *Klebsiella* and *E. coli* from reptiles was resistant to tetracycline.

1. Introduction

A small survey of the faeces of reptiles on the Krakatau Islands and West Java was done to determine their enteric bacterial flora and the antibiotic resistance of the bacteria. The results are compared with those from a larger survey of mammalian faecal flora made during the same expedition (Graves *et al.* 1988).

The survey involved 19 individual reptiles of six species: *Hemidactylus frenatus* (house gecko), *Gekko gecko* (tokay), *Gekko monarchus* (king gecko), *Mabuia multifasciata* (kadal skink), *Chrysopelea paradisi* (paradise tree snake) and *Varanus salvator* (monitor).

An introductory paper to this series (Thornton & Rosengren 1987) discusses the environment and the geological and biotic history of islands of the Krakatau group in the context of studies of recolonization and community succession.

2. Materials and methods

The work was done during the 1985 La Trobe University Zoological expedition to the Krakatau Islands.

(*a*) *Faecal samples*

All samples were taken immediately after the capture of reptiles by P. A. Rawlinson or D. A. McLaren. The reptiles were alive at the time of sampling. The skin surrounding the cloaca was vigorously cleaned with 70% isopropyl alcohol by using a hospital skin cleansing swab, to prevent skin bacteria from contaminating the sample. The sampling swab, the same type as that used for mammals (Graves *et al.* 1988), was inserted approximately 2 cm into the cloaca of each reptile and rotated several times to ensure the transfer of faecal material on to the swab, which was immediately transferred to Stuart's transport medium and stored at ambient temperature (approximately 30 °C) for between 2 and 5 weeks (refrigeration was not available).

(*b*) *Analyses of samples*

The microbiological analysis was identical to, and done at the same time as, the mammalian faecal analysis (see Graves *et al.* 1988).

Either the χ^2 test or Fisher's exact probability test was used to compare data, depending on the sample size.

3. Results

(*a*) *Faecal flora of reptiles*

A total of 23 individual reptiles was sampled but from four of these the cloacal swabs were sterile on subsequent microbiological analysis, indicating that bacteria had not survived in Stuart's transport medium. These four samples were all from *Hemidactylus frenatus* specimens on Java (2), Sertung (1) and Anak Krakatau (1). The faecal bacterial flora from the eight non-sterile swabs of *Hemidactylus frenatus* did not appear to be very different from those from other gecko species. However, data for this species, and possibly others, may over-represent the faecal bacteria able to survive the sampling treatment and storage.

The most prevalent bacterium was a species of *Citrobacter*, detected in 17 out of 19 individual reptiles, followed by species of *Enterobacter* (8 out of 19 individuals), *Klebsiella* (5 out of 19) and

Pseudomonas (5 out of 19). A few reptiles also contained *Escherichia coli*, *Aeromonas* sp. or *Proteus/Providencia* sp. (table 1).

The only obvious difference between the bacterial spectra of the faecal samples examined was the absence from the three gecko species (*G. gecko*, *G. monarchus* and *H. frenatus*) of species of *Pseudomonas*. There was no obvious difference in floral spectrum between reptiles taken from the various islands of the Krakatau group. However, the few individuals sampled indicates the need for caution in the interpretation of our results.

Table 1. Enteric bacteria of reptiles on Java and the Krakatau Islands

species of reptile	reptile code number	enteric bacteria: *Citrobacter*	*Enterobacter*	*Klebsiella*	*Pseudomonas*	others
Hemidactylus frenatus	5J	X	·	·	·	
	67R	X	X	X	·	
	68R	X	·	·	·	
	75R	X	·	·	·	
	53S	X	·	X	·	*Escherichia coli*
	54S	X	X	·	·	
	28A	X	·	·	·	
	30A	·	X	·	·	
Gekko gecko	4J	X	·	·	·	*Aeromonas*
	7J	X	·	X	·	*Escherichia coli*
Gekko monarchus	76R	·	·	X	·	
Mabuia multifasciata	64R	X	X	·	X	*Aeromonas*
	65R	X	X	X	X	
	66R	X	·	·	·	
Chrysopelea paradisi	41P	X	X	·	·	*Proteus/Providencia*
	56S	X	X	·	X	
	31A	X	·	·	X	
Varanus salvator	73R	X	·	·	X	
	74R	X	X	·	·	
totals	19	17/19	8/19	5/19	5/19	

J, Java; R, Rakata; S, Sertung; P, Panjang; A, Anak Krakatu; X, this bacterium isolated from the specified reptile.

Sufficient numbers of species of *Citrobacter* were isolated to enable analysis of their antibiotic-resistance patterns (table 2). There was no obvious difference in these patterns between samples from different islands. A comparison of the patterns between reptile species suggested differences, although these were not statistically significant. For example, from three individuals of *C. paradisi*, four isolates of *Citrobacter* were made; none was resistant to chloramphenicol. In contrast, from two individuals of *V. salvator* four isolates were all resistant to chloramphenicol (table 2). Given the small numbers of individuals sampled, however, this difference cannot be considered biologically significant. None of the reptiles, including the three specimens from Java, harboured *Streptococcus faecalis* (table 1). None of the 10 *Klebsiella* isolates (including two from Javan reptiles) were significantly antibiotic resistant and none was resistant to tetracycline (table 3). The two *E. coli* isolates were fully sensitive to the antibiotics tested (table 3).

Table 2. Comparison of antibiotic-resistance patterns of *Citrobacter* from cloacal swabs of reptiles on Java and the Krakatau Islands

site	reptile code number	species of reptile	number of different *Citrobacter* isolated from reptiles†	antibiotic-resistance pattern	average number of antibiotic-resistance factors per *Citrobacter* isolate	
Java	5J	*Hemidactylus frenatus*	2	Cep^{r} Amp^{r}; Cep^{r}		1.60‡
	4J	*Gekko gecko*	2	Cep^{r} Amp^{r}; Cep^{r}		
	7J	*Gekko gecko*	1	Cep^{r}; Chl^{r}		
Rakata	67R	*Hemidactylus frenatus*	3	Cep^{r}; Chl^{r} Amp^{r}; Cep^{r}; Chl^{r} Amp^{r}; Cep^{r}; Sul^{r}; Chl^{r}	2.0	1.96‡
	68R	*Hemidactylus frenatus*	1	Cep^{r}		
	75R	*Hemidactylus frenatus*	1	Amp^{r}; Cep^{r}; Chl^{r}		
	64R	*Mabuia multifasciata*	1	Cep^{r}		
	65R	*Mabuia multifasciata*	2	Amp^{r} Cep^{r}; Chl^{r}		
	66R	*Mabuia multifasciata*	4	fully sensitive Cep^{r} Chl^{r} Cep^{r}; Chl^{r}		
	73R	*Varanus salvator*	1	Cep^{r}; Chl^{r}		
	74R	*Varanus salvator*	3	Cep^{r}; Chl^{r} Amp^{r}; Cep^{r}; Chl^{r} Amp^{r}; Cep^{r}; Sul^{r}; Chl^{r}		
Panjang	41P	*Chrysopelea paradisi*	1	fully sensitive	0.0	
Sertung	56S	*Chrysopelea paradisi*	1	Amp^{r}; Cep^{r}; Sul^{r}	2.2	
	53S	*Hemidactylus frenatus*	3	Cep^{r} Amp^{r}; Cep^{r} Amp^{r}; Cep^{r}; Sul^{r}		
	54S	*Hemidactylus frenatus*	1	Amp^{r}; Cep^{r}		
Anak Krakatau	28A	*Hemidactylus frenatus*	1	Amp^{r}; Cep^{r}; Chl^{r}	2.0	
	31A	*Chrysopelea paradisi*	2	Cep^{r} Amp^{r}; Cep^{r}		

† Includes *C. freundii*, *C. diversus* and unspeciated isolates. Amp^{r}, resistant to ampicillin; Cep^{r}, resistant to cephalothin; Sul^{r}, resistant to sulphamethoxazole; Chl^{r}, resistant to chloramphenicol; ‡ difference not significant.

Table 3. Comparison of antibiotic-resistance patterns of *Klebsiella* and *Escherichia coli* from reptiles on Java and the Krakatau Islands

bacterium	location	reptile code number	species of reptile	number of different bacterial isolates from reptiles	antibiotic-resistance pattern	average number of antibiotic-resistance factors per bacterial isolate
Klebsiella	Java	7J	*Gekko gecko*	2	fully sensitive Cep^r	1.0
	Rakata	76R	*Gekko monarchus*	3	fully sensitive Amp^r Amp^r; Sul^r	
	Rakata	65R	*Mabuia multifasciata*	2	fully sensitive Amp^r	
	Rakata	67R	*Hemidactylus frenatus*	2	Amp^r Amp^r; Sul^r	
	Sertung	53S	*Hemidactylus frenatus*	1	Amp^r; Sul^r	
Escherichia coli	Java	7J	*Gekko gecko*	1	fully sensitive	0.0
	Sertung	53S	*Hemidactylus frenatus*	1	fully sensitive	

Table 4. Comparison of bacterial faecal flora of reptiles and mammals on Java and the Krakatau Islands: percentage of animals excreting the specified bacterium

(Mammal data from Graves *et al.* (1988).)

animals†	*Citrobacter*	*Enterobacter*	*Klebsiella*	*Pseudomonas*	*Escherichia coli*
reptiles‡ (19)	89%	42%	26%	26%	11%
mammals§ (59)	37%	46%	44%	7%	64%
difference	$p < 0.05$	n.s.	n.s.	$p < 0.05$	$p < 0.05$

† Number of animals sampled in parentheses.

‡ Includes *Hemidactylus frenatus*, *Gekko gecko*, *Gekko monarchus*, *Mabuia multifasciata*, *Varanus salvator* and *Chrysopelea paradisi*.

§ Includes bats (*Myotis muricola muricola*, *Cynopterus tittaecheilus tittaecheilus*, *C. brachyotis javanicus* and *C. sphinx angulatus*) and rats (*Rattus rattus* and *R. tiomanicus*).

Table 5. Comparison of *Citrobacter* from reptiles and mammals on Java and the Krakatau Islands: resistance (Chl^r) or sensitivity (Chl^s) to chloramphenicol

percentage of *Citrobacter* (numbers in parenthesis) isolated from:

	reptiles	mammals
Chl^r	43% (13)	8% (2)
Chl^s	57% (17)	92% (23)

$P < 0.01$.

(*b*) *Comparison of the faecal flora of reptiles and mammals*

The data for mammals are presented in a preceding paper (Graves *et al.* 1988) of this series.

Overall, reptiles harboured species of *Citrobacter* and *Pseudomonas* more frequently, and *E. coli* less frequently, than did mammals ($p < 0.05$) (table 4). Only the *Citrobacter* species were isolated in sufficient numbers from both reptiles and mammals for a comparison to be made of antibiotic-resistance patterns. The *Citrobacter* species of reptiles were more resistant to chloramphenicol than were those of mammals ($p < 0.01$) (table 5).

4. Discussion

This small survey of the faecal bacteria of reptiles on Java and the Krakatau Islands was done as an adjunct to a more extensive survey of mammalian faecal flora done at the same time (Graves *et al.* 1988). Conclusions are limited because only a few individual reptiles were sampled (19 viable swabs), but nevertheless the survey may act as a baseline for further studies.

The study demonstrated that *Citrobacter* species (*C. freundii* and *C. diversus*) were the predominant faecal bacteria of reptiles and that in this respect reptiles differed from mammals. It is interesting to speculate as to what aspect of reptilian gut physiology favours *Citrobacter* species rather than *E. coli*, which is ubiquitous in mammals. Possibly the periodically lower body temperatures of reptiles, or simply a different diet, are involved.

The higher proportion of *Pseudomonas* in some reptiles (*M. multifasciata*, *C. paradisi* and *V. salvator*) may also be significant, but the basis for this is also unknown. No *Pseudomonas* were detected in samples from the three gecko species, indicating possible differences between the faecal flora of different reptile groups. The geckos are not intimately associated with soil, which harbours *Pseudomonas* species; the skink and monitor are ground-dwelling and the latter is a carrion-eater, and they may be more exposed to soil bacteria than are geckos. The paradise tree snake, although tree-dwelling, does come to ground (often by 'gliding').

No attempt was made in this study to isolate the possible low levels of *Salmonella* species in faecal specimens. *Salmonella* species have been detected previously in geckos by using enrichment cultures (Oboegbulem & Iseghohimhen 1985).

H. frenatus, the house gecko, appears to have a different faecal flora from the other reptiles because it was from this species only that some faecal swabs failed to grow any bacteria. Four out of twelve *Hemidactylus* swabs failed to produce bacteria. However, no conclusions can be drawn concerning the faecal flora of this species; even those swabs that were productive may have lost one or more bacterial species during the storage period in transport medium.

On the Krakataus, none of the mammals harboured *Streptococcus faecalis*; nor did any of the reptiles on the islands. However, because only three individual reptiles were successfully sampled on Java, it was not possible to determine if any of the Javan reptiles harboured this bacterium, as did some of the Javan mammals.

The antibiotic-resistance patterns of the *Citrobacter* species from reptiles differed from those of the mammalian *Citrobacter* species, chloramphenicol resistance being more frequent in reptilian isolates. Resistance was found in 43% of the strains isolated from reptiles, resistant strains occurring in five of the six reptile species whereas only 8% of the strains isolated from mammals were resistant, occurring in only one of the mammal species (*R. tiomanicus*). This is probably related to differences in diet. In the mammalian study, more chloramphenicol-resistant *E. coli* were detected from rats on Panjang than elsewhere on the Krakatau Islands. Only a single reptile specimen (*C. paradisi*) was sampled from this island, and its *Citrobacter* species was fully sensitive.

In the mammalian study, no tetracycline-resistant *E. coli* or *Klebsiella* were detected on the Krakatau Islands, although several tetracycline-resistant isolates were obtained from mammals on Java. None of the reptiles harboured tetracycline-resistant *E. coli* or *Klebsiella*, although the small numbers of individuals sampled precludes a comparison between Javan reptiles and those from the Krakatau Islands.

This small survey has suggested some differences between the faecal flora of reptiles and mammals, and possibly between different reptile groups, on the Krakatau Islands. A more extensive survey is necessary to confirm or refute these suggested differences.

References

Graves, S. R., Kennelly-Merrit, S. A., Tidemann, C. R., Rawlinson, P. A., Harvey, K. J. & Thornton, I. W. B. 1988 Antibiotic-resistance patterns of enteric bacteria of wild mammals on the Krakatau Islands and west Java, Indonesia. *Phil. Trans. R. Soc. Lond.* B **322**, 339–353. (This volume.)

Oboegbulem, S. & Iseghohimhen, A. 1985 Wall geckos (Geckonidae) as reservoirs of *Salmonella* in Nigeria: problems for epidemiology and public health. *Int. J. Zoon.* **12**, 228–232.

Thornton, I. W. B. & Rosengren, N. J. 1988 Zoological expeditions to the Krakatau Islands, 1984 and 1985: general introduction. *Phil. Trans. R. Soc. Lond.* B **322**, 273–316. (This volume.)